Reports and Letters

Structure-Toxicity in Hexachlorocyclohexane Isomers

The remarkable differences in the physiological effects of the various isomers of hexachlorocyclohexane (BHC) raise the question of whether these actions are related to specific biochemical reactions, or whether they may be inferred from certain physical considerations (*1*). From the standpoint of both molecular structure and physiological activity, the greatest difference in isomers is between the β- and γ-forms. The former has a relatively plane shape and only very weak physiological activity as a depressant, while the γ-form has a relatively spherical shape and strong convulsant (insecticidal) action. Table 1 shows the configuration, size, and action of the various isomers.

If it is assumed that both β- and γ-isomers act on the cell membrane, can one explain the different types of action of these and other BHC isomers using an arbitrary but identical model of the membrane in all cases? A membrane composed of cylindrical lipoprotein macromolecules aranged in regular hexagonal packing may represent a simple, generalized model of the actual structure in living cells. The interspaces between these macromolecules are then the "pores" of the membrane, into which various solutes can be introduced. The size of these interspaces is a function of the diameter of the membrane molecules and their separation from one another. If we set the interspace diameter as just slightly larger than the diameter (8.5 A) of the γ-BHC isomer (as measured in the plane of the cyclohexane ring), we thereby exclude all penetration of the β-isomer (9.6 A) in a plane orientation. Considerations of geometry show, however, that the β-isomer will fit into the interspace if the molecule is turned on end (we shall refer to the two orientations of this and other isomers as plane and end-on); thus, the difference between these two isomers can be expressed in terms of their orientations in the membrane.

The usual effect of filling up the membrane interspaces with small molecules is that of narcosis, or the depression of excitable tissue (*2*), an effect that is largely independent of molecular shape. Such a situation is understandable on the basis of this model, because, as long as a molecule is much smaller than the diameter of a circle that can be inscribed in the interspace, there is no orientational barrier to its penetration. For the process of narcosis we may expect that it is the thermodynamic activity of a substance that is important in determining the relative magnitude of the narcotic effects (*3*). The thermodynamic activities of the pure solid isomers (with the liquid as the standard state) will be inversely proportional to their melting points and heats of fusion, and they can be approximated by the solubilities of the isomers in paraffinic solvents (*4*). The order of such solubilities is $\delta > \gamma > \alpha > \beta$, with δ about 100 times as soluble as β. Hence, the δ-isomer, which, like the β-isomer, is excluded from a plane orientation in the membrane, can be expected to be a strong depressant, and the β-isomer can be expected to be a weak one.

The fit of the various isomers into a membrane interspace of an arbitrarily chosen size (*5*) is shown in Fig. 1. Since small molecules, although quite capable of causing narcosis, do not produce membrane excitation, we might conclude that the only requirement for excitation is to make a molecule large enough. If, however, a molecule is made very much larger than the mean membrane interspace size, the probability of its entering the membrane becomes vanishingly small (at least by the process of interspace penetration). If molecules of a size comparable to that of the interspace are introduced into the membrane, the following considerations would seem important in determining the effects that are to be produced: (i) the strength of the attractive forces between the molecules of the substance being introduced and the membrane molecules, and (ii) the strength of the attractive forces between the membrane molecules themselves.

If both of these forces are of a comparable order of magnitude, the molecule should remain in the interspace without distortion of the membrane structure. If the inserted molecule has regions of strong attractive force that interact with the membrane molecules, it would appear possible that it could distort the mean position of the membrane molecules around the interspace. Since the stability of the membrane is determined by the position of its molecules with respect to one another, distortion of the position of these molecules may lead to local regions of instability of the structure and to ion leaks that are a prelude to excitation. In the case of the BHC isomers, strong attractive forces are associated with the chlorine atoms, and membrane distortion can be considered possible when a maximal number of these can interact with membrane molecules in such a way that they disturb the equilibrium position of the membrane molecules.

From Fig. 1 we note that plane orientations are not possible for the α-, β-, δ-, and ε-isomers, although end-on orientations are possible for all forms. It is suggested that, although the γ-isomer may penetrate in many orientations, it can rotate after penetration, and the attractive forces of its chlorine atoms will be best satisfied when a plane orientation in the interspace is assumed. Reorientation of the other isomers listed here will not be possible for steric reasons.

The reason suggested as to why end-on orientations of the isomers result in narcosis, rather than excitation, is that in this orientation the molecules do not in general fit the interspace very closely. They therefore have a tendency to be

Table 1. Configuration, size, and action of isomers

Isomer	Melting point (°C)	Configuration*	Molecular diameters† in plane of ring			Molecule thickness	Physiological effect
β	297	*eeeeee*	9.5	9.5	9.5	5.4	Inert or weak depressant
δ	130	*peeeee*	8.5	9.5	9.5	6.3	Strong depressant
α	157	*ppeeee*	8.5	8.5	9.5	7.2	Weak excitant
γ	112	*pppeee*	8.5	8.5	8.5	7.2	Strong excitant
ε	219	*peepee*	7.5	9.5	9.5	7.2	Not insecticidal
η	90	*peppee*	7.5	9.5	8.5	7.2	Not insecticidal
θ	124?	*pepeee*	8.5	9.5	8.5	6.3	Unknown

* The notation is such that *e* (equatorial) represents a chlorine atom located approximately in the plane of the cyclohexane ring; *p* (polar) represents chlorine atoms distributed alternately above and below the plane of the ring [see H. D. Orloff, *Chem. Rev.* 54, 347 (1954)].

† Insofar as the scale of Stuart models is accurate, these measurements can be considered in angstrom units. The three values for diameters in the plane of the ring are at intervals of 60° each.

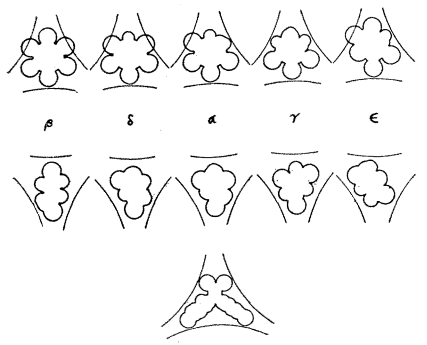

Fig. 1. Scale drawings from Stuart models showing the fit of various BHC isomers into a membrane interspace for plane (top) and one end-on (middle) orientation. The interspace (shown by curved lines) is formed by three cylindrical membrane molecules with a diameter of 40 A at a separation from each other of 2 A. All plane orientations, except that of γ-BHC, are excluded, although end-on orientations of all the isomers are permitted. At the bottom the only possible orientation of DDT is shown.

attracted to only one of the three surrounding membrane molecules, thus forming an interspace block but not a distortion.

If the foregoing model is to have any generality, it ought to be capable of accounting for the action of DDT and its various analogs. Diphenyltrichloroethane is capable of occupying the interspace in several orientations, one of which is with one benzene ring parallel to the surface, and another is with both benzene rings in an end-on orientation. The former orientation is not one in which the attractive forces of the halogen atoms can best be made effective, whereas the latter makes use of the attractive forces of not only the halogens but also the two benzene rings. To direct the orientation of diphenyltrichloroethane to end-on, *p,p'*, and, to a lesser extent, *m,m'* substitution with some group of the approximate size of chlorine is effective. If the *p,p'* substituent is made too large (for example, iodo or propoxy), penetration will be delayed or inhibited. By increasing the distance across the molecule (from the chlorine of trichloroethane to *p*-Cl), *p,p'* substitution makes it impossible for DDT to penetrate other than in an end-on orientation, as is shown at the bottom of Fig. 1.

Penetration in this orientation depends very much upon having the two benzene rings in an approximately end-on orientation. The free rotation of the benzene rings that is possible in *p,p'*-dichlordiphenylethane is constrained by 1,1,1-trichloro substitution in the ethane nucleus (*6*). This orientation of the two rings can be disturbed by a number of changes in the DDT molecule, such as by chlorination of the 2 position in ethane and by *ortho* chlorination of either of the benzene rings, changes that tend to rotate the benzene rings with respect to each other. These compounds are all much less active than DDT. Subtraction of halogen from the ethane nucleus is also capable of disorienting the rings, although this becomes important only when two or three halogen atoms have been removed. The change from DDT to dichlorodiphenyldichloroethylene increases markedly the distance between the chlorine atoms of dichloroethylene and their corresponding benzene rings (because of the change in bond angles), and hence makes the molecule nonpenetrating. The dichloroethylene compound is also relatively inactive. The steric situation responsible for the toxicity of DDT would thus appear to involve two factors: (i) a specific orientation of the two benzene rings with respect to each other, and (ii) a distance along the two axes of the molecule (ethane Cl to *p*-Cl) great enough to prevent a plane orientation of one of the rings in the interspace.

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References and Notes

1. This work was aided by a grant (B-139) from the National Institute for Neurological Diseases.
2. L. J. Mullins, *Chem. Rev.* **54**, 289 (1954).
3. J. Ferguson, *Proc. Roy Soc. (London)* **B127**, 387 (1939).
4. J. H. Hildebrand and R. L. Scott, *The Solubility of Non-Electrolytes* (New York, 1950).
5. Space does not permit the derivation of this interspace size from experimental data on narcosis. In a more extensive paper it will be shown that the interspace size need not be set arbitrarily.
6. A somewhat contrary view is taken by R. Riemschneider [*Z. Naturforsch.* **9**, 95 (1954)] who considers DDT analogs as most effective when free rotation of the benzene rings is possible. What has really been noted here is that ring substitution that tends to disturb the normal orientation of DDT results in less active compounds. This does not account for the usually diminished activity in dichlorodiphenylethane where free rotation is really possible.

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Improved Automatic Microtome

The automatic microtome I described in *Science* [**115**, 649 (1952)] that cuts and mounts serial sections of imbedded biological specimens on a 35mm strip of film base has been made more effective by two changes in the manner of its use: (i) specimens are mounted in tissuemat, and (ii) Mylar film is used so that it is possible to stain the sections after cutting.

Originally the instrument cut doubly imbedded specimens. It is preferable to use tissuemat, because it is routinely employed by many laboratories and because it saves imbedding time. The cutting of tissuemat sections works well under all conditions encountered, and they adhere well to the Mylar film without the use of an adhesive coating when the automatic microtome is carefully adjusted.

Staining previously was done on the tissue *in toto,* largely because of the damage suffered by acetate film in the various solvents. With Du Pont's Mylar film, which is not affected by solvents, we are now able to remove the tissuemat and stain the sections on the film in standard solutions.

Freshly cut sections are coated with a thin celloidin solution 0.5 percent and a thin coat of lacquer. Eastman film lacquer has been found to be excellent for the purpose. This treatment secures the sections to the film so that they do not become displaced in subsequent processing. A strip of 5 ft, or about 80 serial sections, can be wound on the reel of a 35mm developing tank such as the Nikor tank. The tissuemat is then extracted with Xylene, and the regular process is continued, using a series of solutions of alcohol. When the dyeing is completed, and the strip is again in Xylene, it is removed from the tank, and a second piece of Mylar film is placed on top of the strip, using a mounting medium. Longer strips can be used with automatic photographic developing equipment.

Projection or examination under the microscope is facilitated, since ordinary 35mm film-handling equipment can be used to bring successive sections into register. Copies may readily be made with ordinary motion-picture equipment.

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Elemental Sulfur Dust, a Nutrient for Lemon Leaves

Certain citrus growers in California have observed that they were able to pack more first-grade fruit from citrus trees that had been dusted with elemental sulfur dust than from nondusted trees. Losses due to injured peels of fruit unprotected from the sun's direct rays were to be expected, but these were considered negligible when they were compared with the increase in first-grade fruit obtained. It is not known how sulfur dust improved the grade of fruit or what unobserved physiological effects sulfur had. It became apparent to one of us, however, while he was assisting in the preparation of "Standard Values in Nutrition and Metabolism" (*1*), that elemental sulfur applied to foliage of higher plants has not been demonstrated to penetrate the foliage and to enter into the anabolic processes therein.